Patent Application
Docket No. BB.243T
Serial No. 10/564,823

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Examiner	:	Mark Halvorson
Art Unit	:	1642
Applicants	:	Ulrike Stein *et al.*
Serial No.	:	10/564,823
Filed	:	April 18, 2006
Confirm. No.	:	2061
For	:	7a5/Prognostin and Use Thereof for the Diagnostic and Therapy of Tumors

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF ULRIKE STEIN, Ph.D. UNDER 37 CFR §1.132

Sir:

I, Ulrike Stein, hereby declare:

THAT, I am a full professor at the Charité University Medicine, Berlin, and that I have many years of experience in cancer research;

THAT, my qualifications are set forth in more detail in Exhibit 1 (attached hereto);

THAT, I am an inventor on the above-referenced application;

THAT, I, have reviewed the specification, the pending claims, and the Office Action mailed July 20, 2009;

And being thus duly qualified, do further declare as follows:

Our invention provides methods for 1) diagnosing whether a tumor is likely to metastasize; and 2) diagnosing whether a subject has cancer. These methods of diagnosis involve determining levels of 7a5/Prognostin (also called MACC1) expression in a biological sample. The biological sample can be derived from tissue or from a bodily fluid. These methods can be applied to various types of cancer, such as, for example, colon cancer, breast cancer, colorectal cancer, rectal cancer, bone cancer, and gastric cancer.

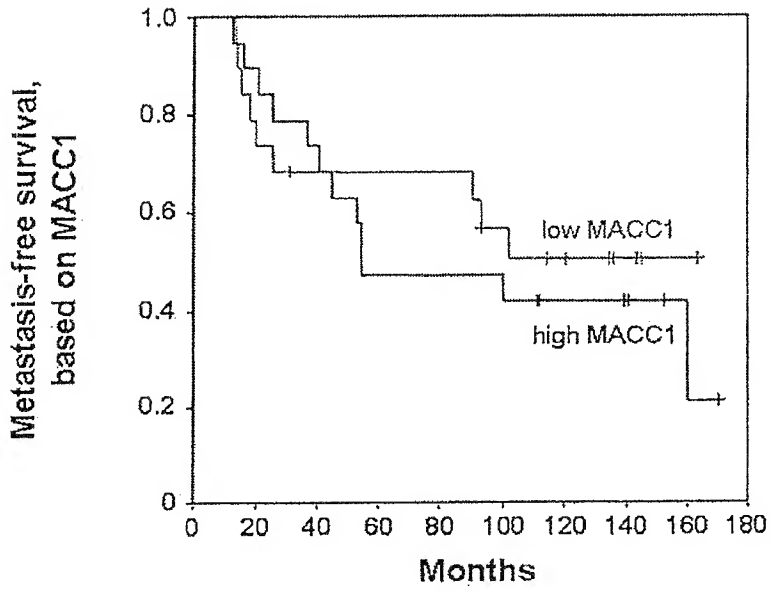
In this Declaration, I report the results of experiments that demonstrate that 7a5/Prognostin is significantly over-expressed in a) cancer patients versus non-cancer patients; and b) cancer patients having tumors that are likely to undergo metastatic spread. The results demonstrate that the 7a5/Prognostin over-expression is a shared characteristic of cancer, particularly metastasizing cancer, regardless of the site of primary tumor origination. In addition, the results show that the over-expression of 7a5/Prognostin can be detected not only in biological samples obtained from the pathological tissue, but also in bodily fluids, such as blood or plasma.

In a first set of experiments, MACC1(7a5/Prognostin) expression levels were evaluated in the tissues of breast cancer patients. Thirty-eight patients diagnosed with mammary carcinoma were analyzed, including three patients with Stage I mammary carcinoma, thirty-four patients with Stage II carcinoma, and one patient with Stage III carcinoma. The patients were within an age group ranging from 39-75, and the median age was 57. Twenty-two patients developed distant metastases during the follow-ups.

During the experiment, tumor cell tissues obtained from patients were snap-frozen. Then, serial cryosections of each tissue were micro-dissected and prepared for RNA isolation. Next, RNA was isolated from micro-dissection tumor cell populations and evaluated for quality. Finally, quantitative RT-PCR was performed to determine MACC1(7a5/Prognostin) expression levels.

The results show that MACC1(7a5/Prognostin) was expressed in all primary breast tumors but that the level of expression varied. As shown in Figure 1, low levels of MACC1(7a5/Prognostin) expression in the primary, not yet metastasized, tumor cells were associated with a more advantageous metastasis-free survival; whereas a high MACC1(7a5/Prognostin) level in the primary tumor indicates a high probability that the primary tumor will metastasize.

Figure 1. Metastasis-free Survival based on MACC1



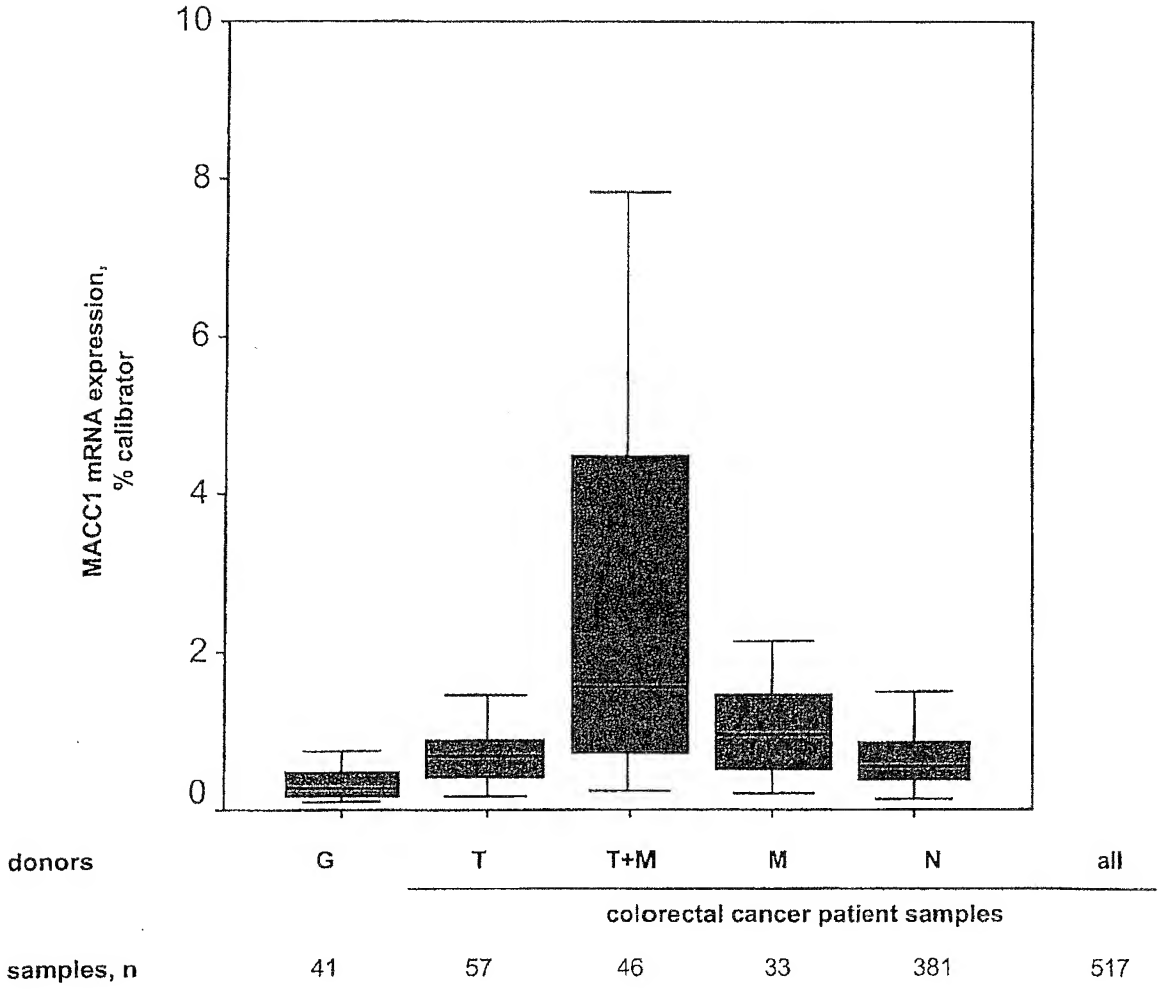
Therefore, the experiments showed that the expression of MACC1(7a5/Prognostin) is higher in malignant tissues (primary tumors) than in corresponding healthy tissues. In addition, in comparison to non-metastasizing primary tumors, primary tumors that will metastasize or have already undergone metastatic spread exhibit a higher level of MACC1(7a5/Prognostin) expression. Accordingly, MACC1(7a5/Prognostin) is a diagnostic, as well as a prognostic, marker for the metastatic potential of cancer.

It is also well recognized that circulating nucleic acids, especially cell-free mRNA, can be detected in plasma. By performing plasma-based expression profiling, tumor-derived mRNA transcripts in blood can be quantified. This technique facilitates the identification of occult tumors in apparently healthy individuals. As a result, such blood-based diagnostics not only provide a useful snap-shot of a patient's biopsy to determine tumor markers, but also the capability of monitoring patient responses and determining the effectiveness of specific therapies.

In a second set of experiments, a non-invasive, reliable, and simple blood-based assay for colorectal cancer (colon cancer and rectum cancer) was performed by quantifying the transcripts of the metastasis promoting gene MACC1(7a5/Prognostin). The results, as shown in Figure 2

below, demonstrate that patients having tumors with synchronous metastasis exhibited significant over-expression of MACC1. In addition, MACC1 is highly over-expressed in metachronous metastasis. Hence, the blood assay technique in this experiment can be used to diagnose the existence of occult tumors and/or the development of metastasis in apparently healthy individuals as well as those newly diagnosed or already treated patients. Additionally, the blood assay can also be used to identify the specific disease stage of each cancer patient.

Figure 2. MACC1 mRNA Expression, % Calibrator



Donors	
H	healthy volunteers
T	tumour
T+M	tumor with synchronous metastasis (first diagnosis)
M	metachronous metastasis (first diagnosis)
N	Nachsorge (Follow-up)

In a third set of experiments, we obtained and analyzed blood samples of about 500 cancer patients, in groups of colorectal (Figure 4a,b), sub-groups of only colon (Figure 5a,b) and only rectal (Figure 6), and gastric cancer patients (Figure 7). In the controls, blood samples of two independent cohorts of healthy volunteers (Figure 3) are analyzed. The results show that 7a5/Prognostin is significantly over-expressed in tumor patients as compared to healthy controls, wherein such over-expression is the most significant in UICC stage IV patients, who develop metastases (*see* Figure 8, showing the UICC classification system). Furthermore, the over-expression of 7a5/Prognostin in blood is a shared characteristic for patients with a variety of different cancer types.

Conclusion

In conclusion, these experiments demonstrate that 7a5/Prognostin (also called MACC1) expression can be used for 1) diagnosing whether a tumor is likely to metastasize; and 2) diagnosing whether a subject has cancer. The biological sample can be derived from tissue or a bodily fluid. In addition, these methods can be applied to various types of cancer, such as for example, colon cancer, breast cancer, colorectal cancer, rectal cancer, bone cancer, and gastric cancer.

Figure 3

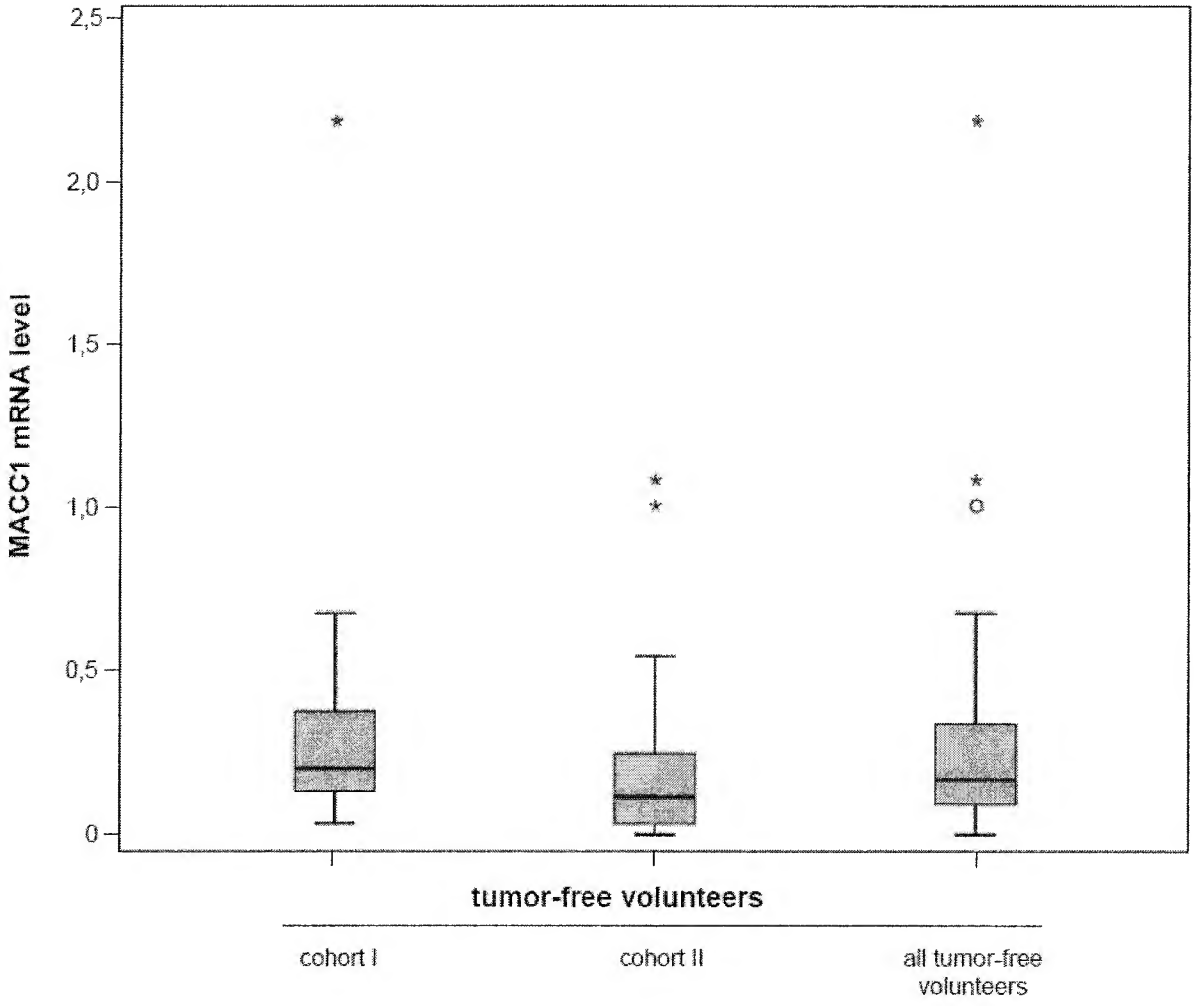

2,5
2,0
1,5
1,0
0,5
0
MACC1 mRNA level
tumor-free volunteers
cohort I
cohort II
all tumor-free volunteers

Figure 4a

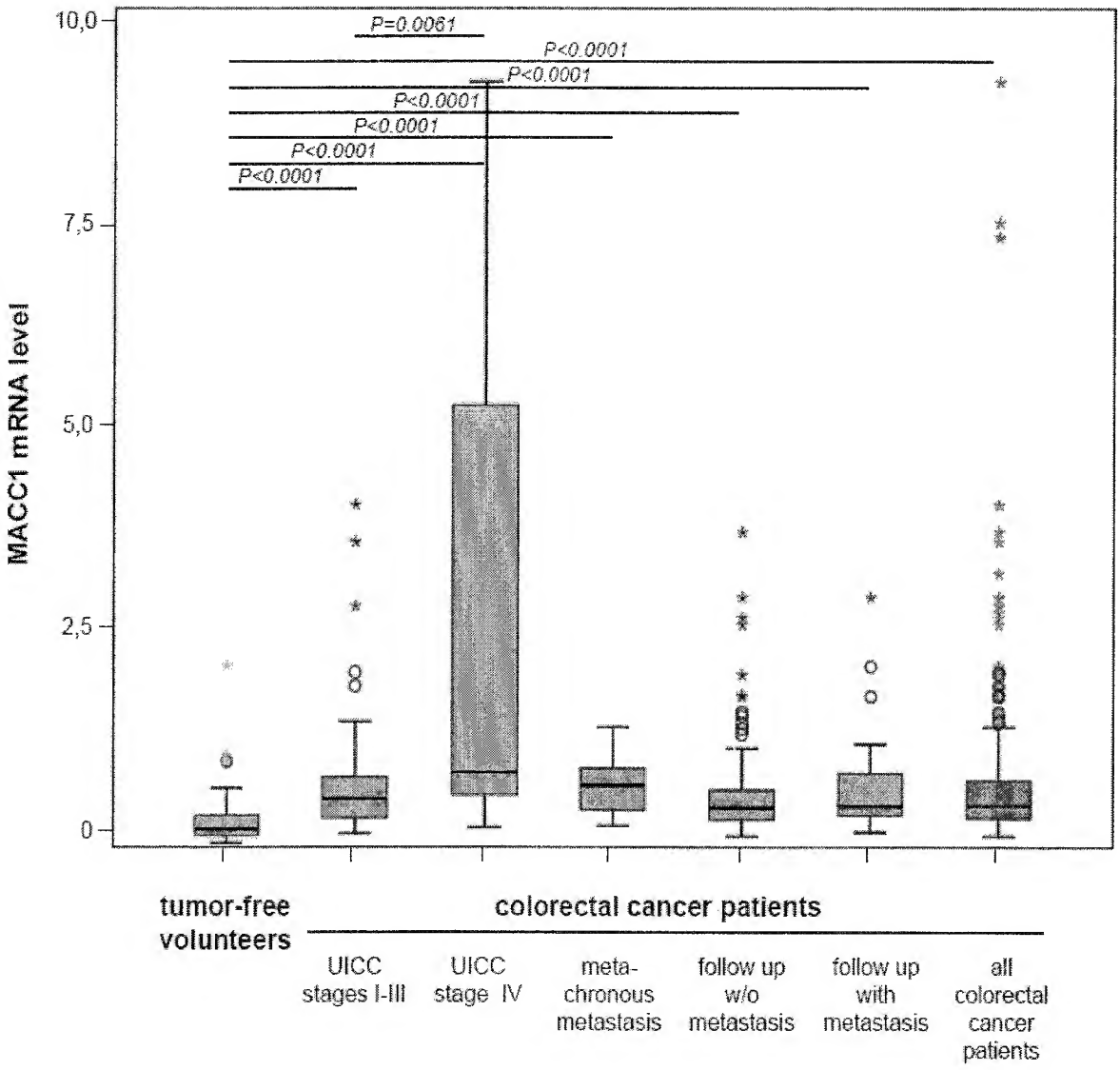

10,0
7,5
5,0
2,5
0
MACC1 mRNA level
P=0.0061
P<0.0001
P<0.0001
P<0.0001
P<0.0001
P<0.0001
P<0.0001
tumor-free volunteers
colorectal cancer patients
UICC stages I-III
UICC stage IV
meta-chronous metastasis
follow up w/o metastasis
follow up with metastasis
all colorectal cancer patients

Figure 4b

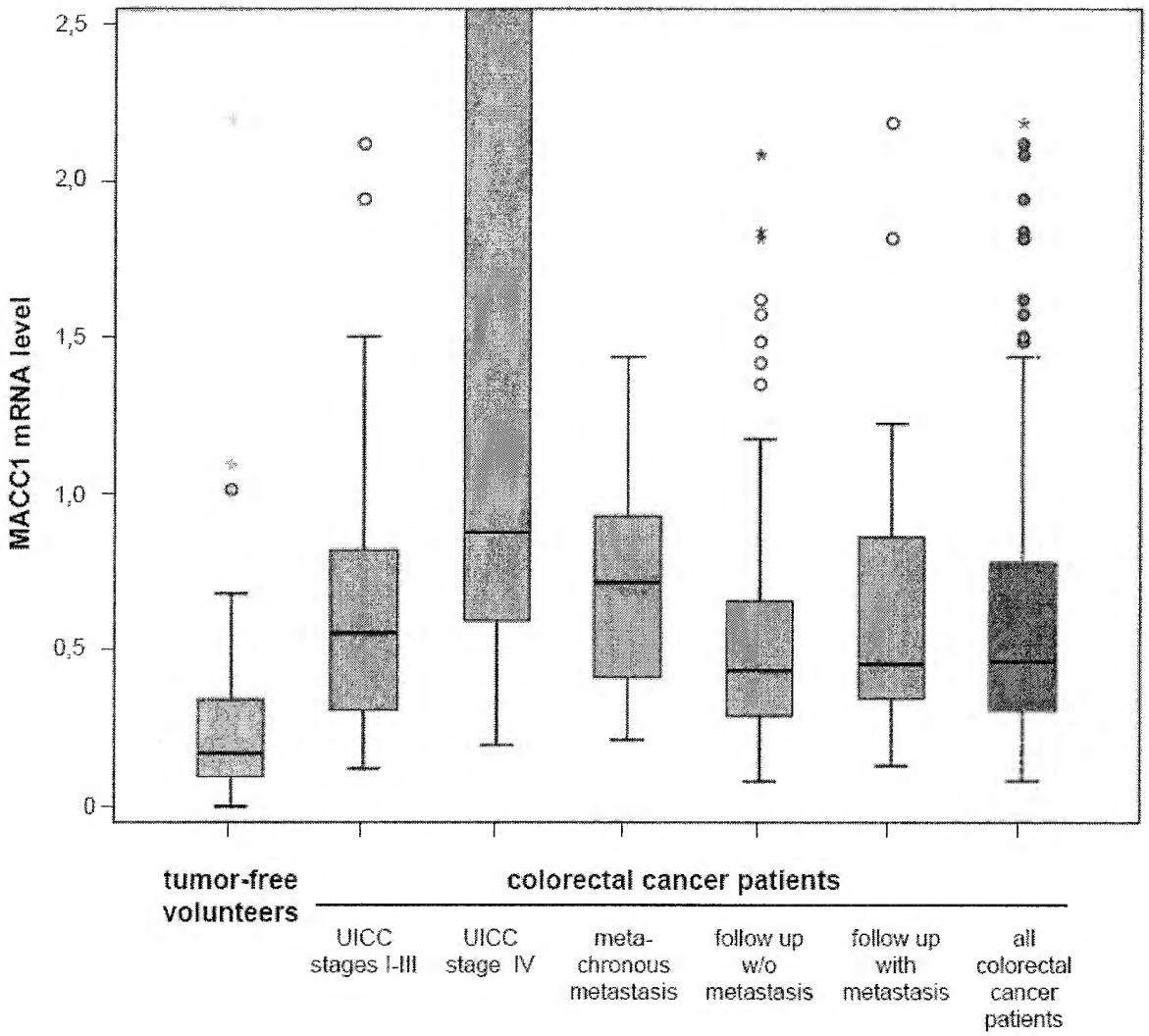

MACC1 mRNA level
2,5
2,0
1,5
1,0
0,5
0
tumor-free volunteers
colorectal cancer patients
UICC stages I-III
UICC stage IV
meta-chronous metastasis
follow up w/o metastasis
follow up with metastasis
all colorectal cancer patients

Figure 5a

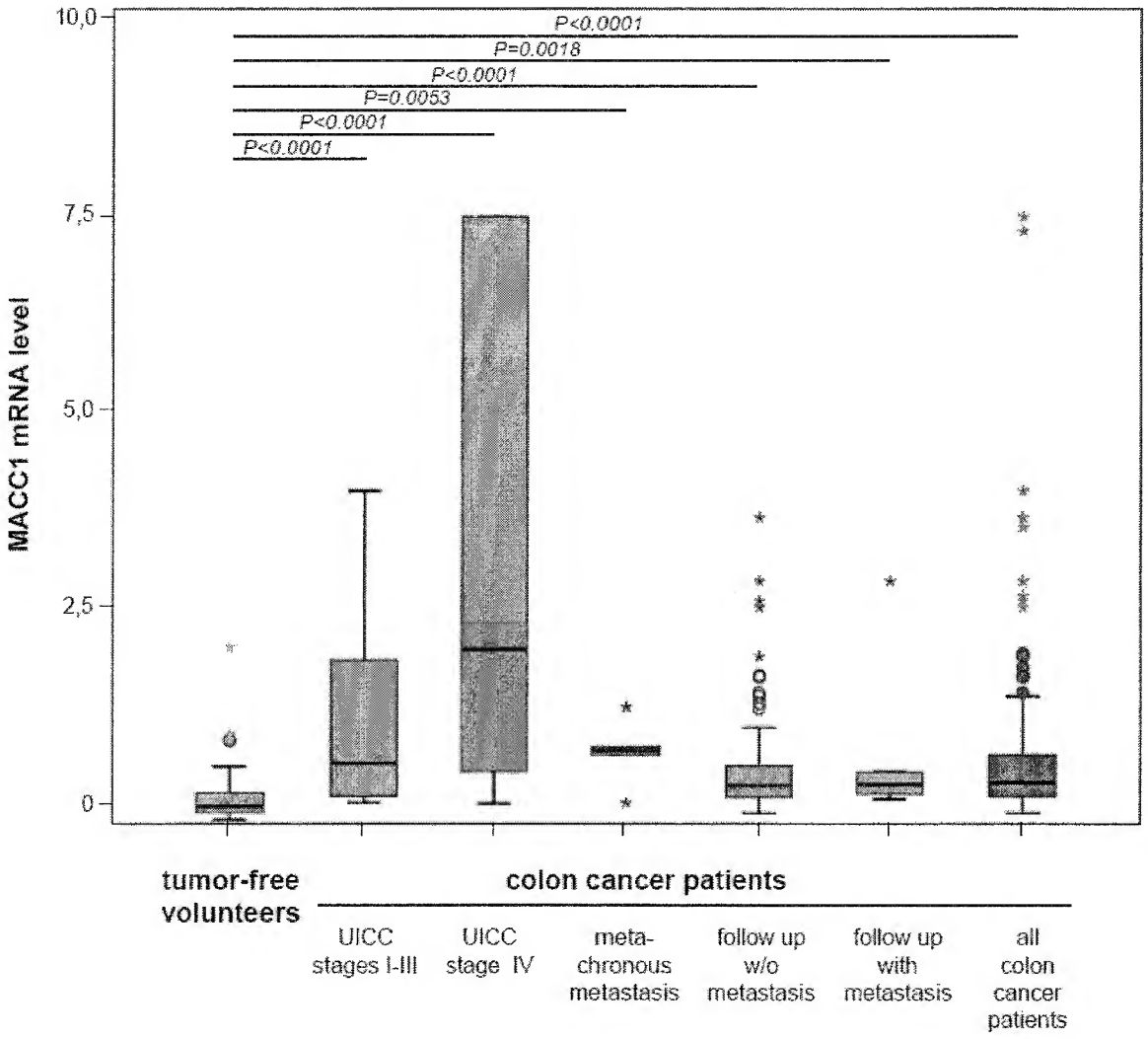

P<0.0001
P=0.0018
P<0.0001
P=0.0053
P<0.0001
P<0.0001
10,0
7,5
5,0
2,5
0
MACC1 mRNA level
tumor-free volunteers
colon cancer patients
UICC stages I-III
UICC stage IV
meta-chronous metastasis
follow up w/o metastasis
follow up with metastasis
all colon cancer patients

Figure 5b

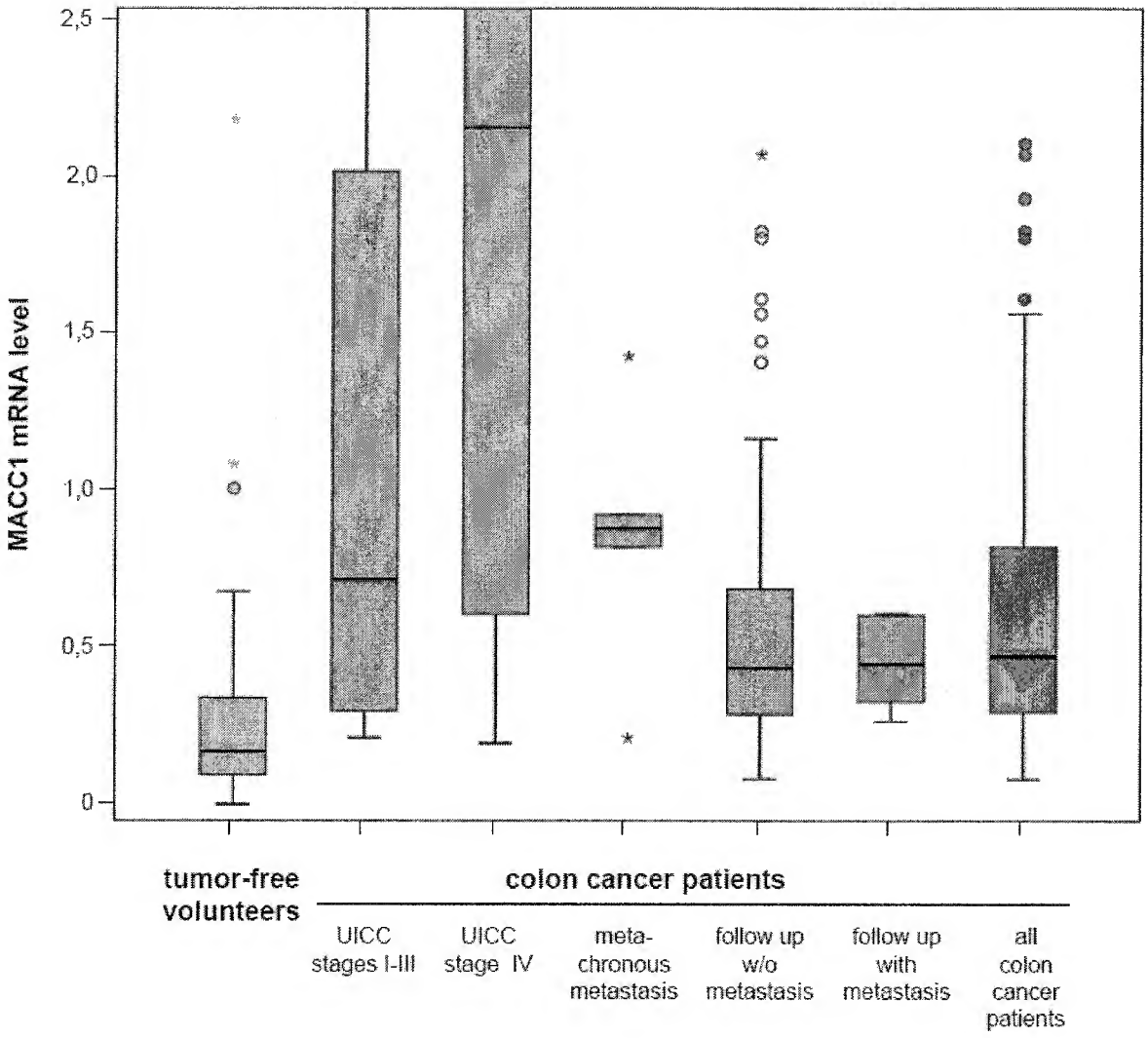

MACC1 mRNA level
2,5
2,0
1,5
1,0
0,5
0
tumor-free volunteers
colon cancer patients
UICC stages I-III
UICC stage IV
meta-chronous metastasis
follow up w/o metastasis
follow up with metastasis
all colon cancer patients

Figure 6

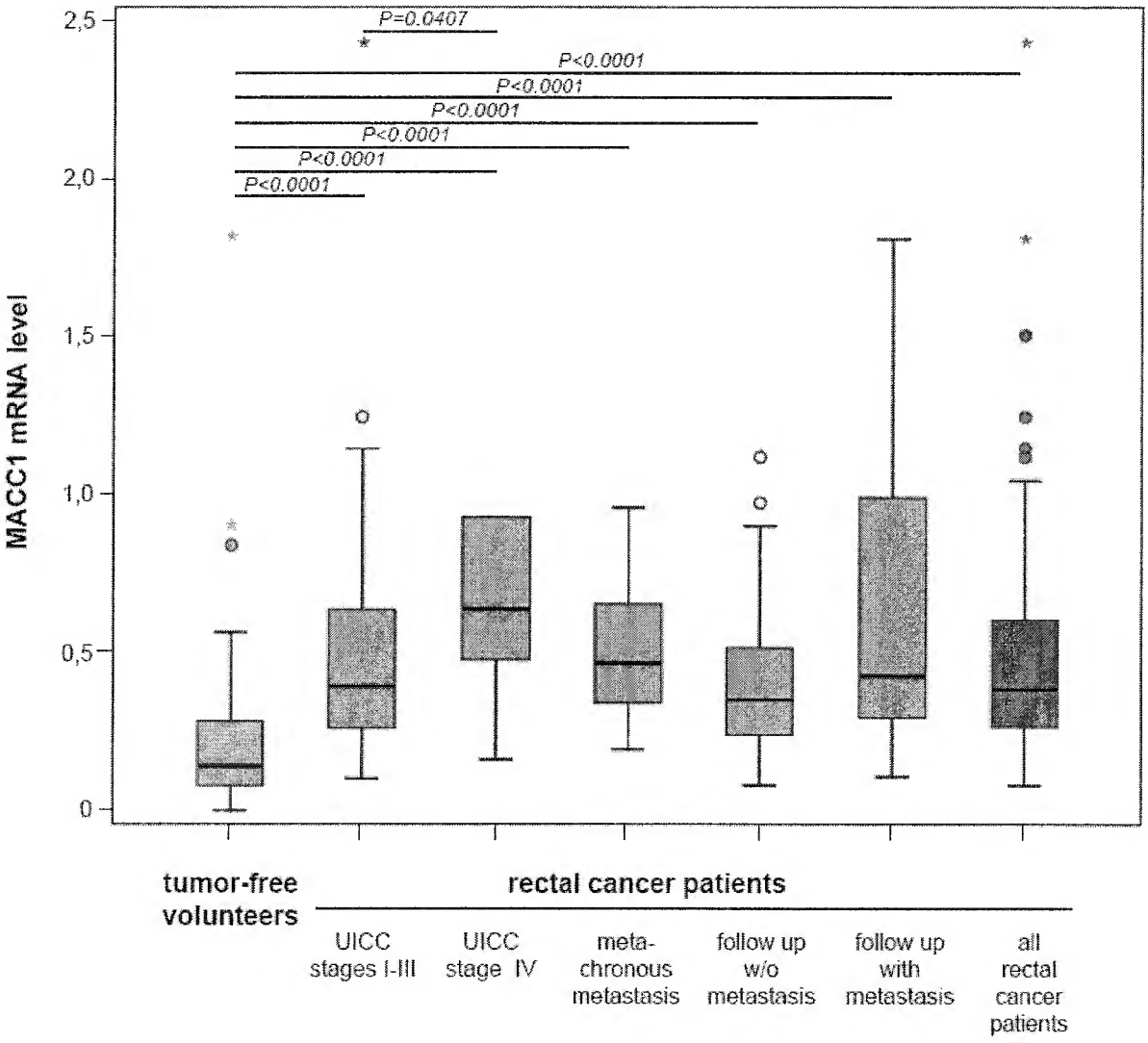

P=0.0407
P<0.0001
P<0.0001
P<0.0001
P<0.0001
P<0.0001
P<0.0001
2,5
2,0
1,5
1,0
0,5
0
MACC1 mRNA level
tumor-free volunteers
rectal cancer patients
UICC stages I-III
UICC stage IV
meta-chronous metastasis
follow up w/o metastasis
follow up with metastasis
all rectal cancer patients

Figure 7

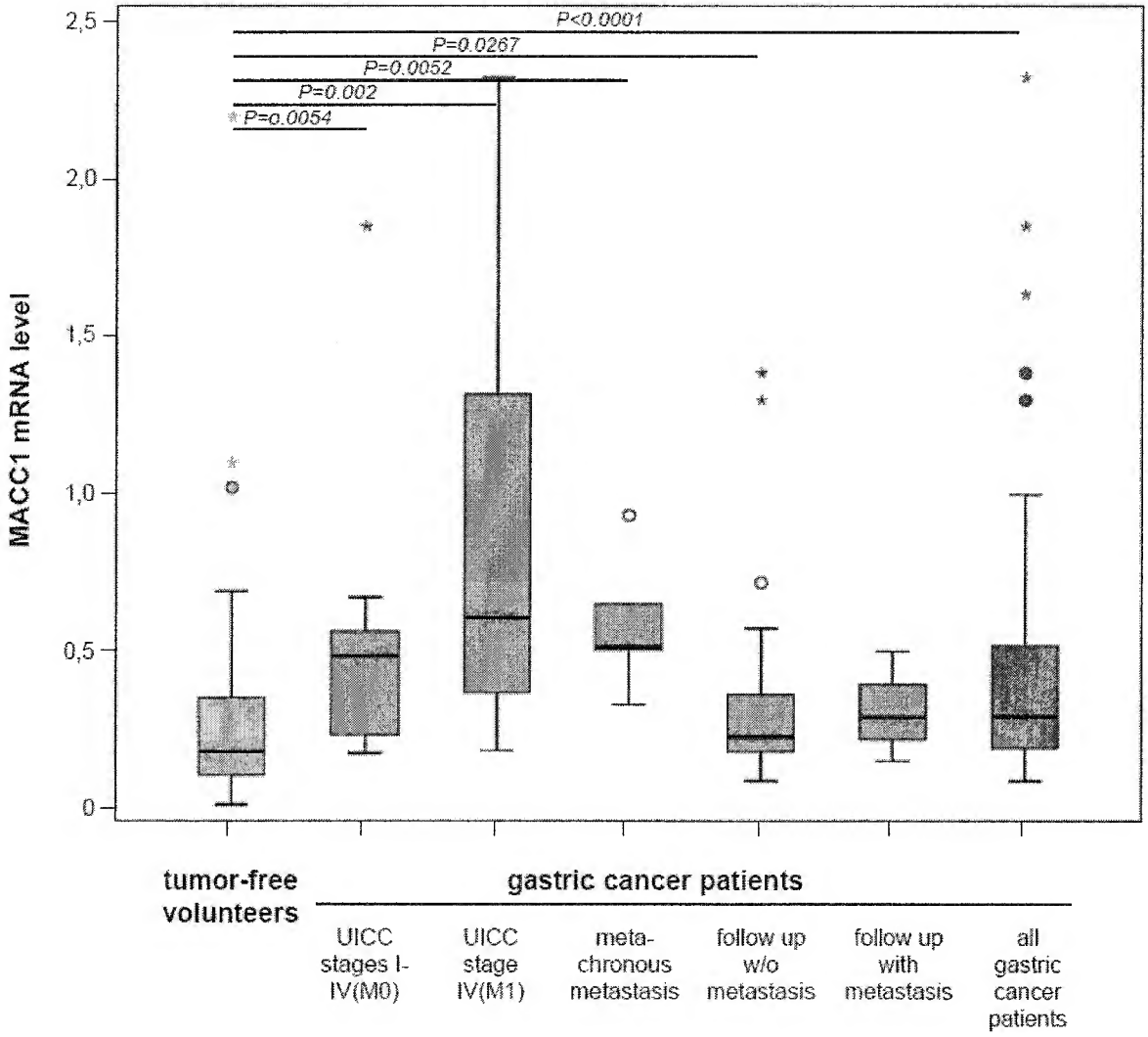


Figure 8

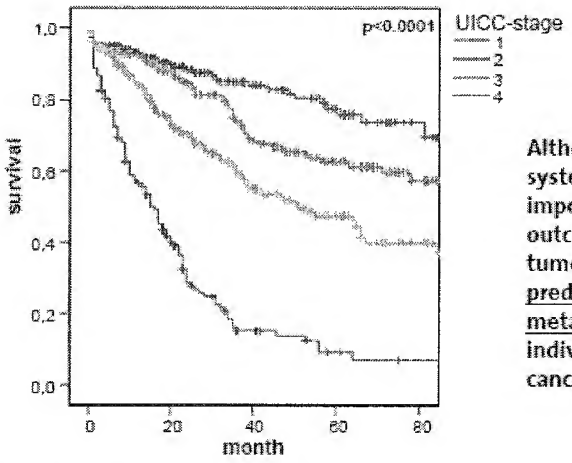
Prediction of Patients Risk

Clinical Problem

Problem: Which patient has a high risk of developing metastases after surgery of primary tumor?

Currently no prediction on metastasis process in colorectal cancer patients possible.

TNM classification[1]
UICC 1: T1-2 N0 M0
UICC 2: T3-4 N0 M0
UICC 3: T1-4 N1 M0
UICC 4: T1-4 N1 M1



Although the TNM system is the most important predictor of outcome for most tumours, there is no prediction on the metastasis process in individual colorectal cancer patients possible

ipal

(1) The TNM (Tumour-Node-Metastasis) classification of the International Union Against Cancer (UICC) is the most widely used system for staging of cancer and the anatomic extent of disease.

I declare further that all statements made in this Declaration of my own knowledge are true and that all statements made on information and belief are believed to be true and further that these statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the specification or any patent issuing thereon.

Dated: 20th January 2010

Ulrike Stein

Attachments: Exhibit 1 (Statement of Qualifications)

EXHIBIT 1

ULRIKE S. STEIN

graduated in Biochemistry, Martin-Luther-University Halle, Germany, 1984, and in Biochemical Medicine, Academy for Medical Education, Berlin, 1991. From 1984-91, she worked at the Central Institute for Cancer Research, Berlin. She completed her PhD thesis in biochemistry, Humboldt-University, Berlin, 1991. She worked from 1992-93 as Post-Doc at the Max-Delbrück-Center for Molecular Medicine (MDC), Berlin.

From 1994-95, she was visiting scientist as Alexander von Humboldt-fellow at the National Cancer Institute, Frederick, MD. She was invited as guest consultant for one month at the NCI in 1996, 1997, 1998, 2001, 2003, and 2007.

She is group leader of the "Tumor Metastasis and Therapy Response" program, at the MDC (1996-2000), at the Robert-Rössle-Tumor Clinic, Charité, University Medicine (2000-2006), and since 2007 at the Experimental and Clinical Research Center, Charité, University Medicine, Berlin, as part of the "Surgical Oncology" department.

She received her Habilitation in biochemistry (Assistant Professorship) in 2003. In 2009, she was appointed as full professor (Associate Professorship) from the Charité University Medicine, Berlin.